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# Causes of variation in sex ratio and modes of sex determination in the Mollusca—an overview*

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**Abstract:** The mechanisms for variation in the primary and apparent sex ratios, from both theoretical and empirical perspectives, are reviewed. A series of experiments on the sex ratios and mode of sex determination in the apple snail *Pomacea canaliculata* (Lamarck, 1822) show that broods have highly variable sex ratios even though the sex ratios of populations are 1:1. I suggest that the mechanism responsible for this pattern is oligogenic sex determination, *i.e.*, sex determination by a small number of genes. Two other molluscan groups, the protandric oysters of the genus *Crassostrea* Sacco, 1897, and mussels of the genus *Mytilus* Linnaeus, 1758 also show variable sex ratios. In both cases, the number of genes responsible for the variation appears to be small.

**Key words:** sex-determining gene, genetic mechanism, genetic sex determination, molluscs, *Pomacea canaliculata*

Fisher (1930) was the first to show that the sex ratio of a population should be 1:1 if producing a son or a daughter requires an equal cost. Because each offspring inherits half of its genetic material from its mother and half from the father, the members of the sex in short supply will have a higher expectancy of genetic contribution to the next generation than members of the sex in excess. Therefore, the genetic tendencies that produce members of the sex in short supply will be selected by natural selection, until an equal sex ratio is realized in the population.

Since Fisher's theory, studies on sex ratio have expanded along three major lines. The first line is the extension of this theory to include cases for which its premises do not hold, such as local mate competition or local resource competition (Hamilton 1967, Trivers and Willard 1973, Clarke 1978). The second is to treat sexuality in general such as sex allocation in simultaneous hermaphrodites (Charnov 1982). The third treats the conflict between individuals or between genes for reproduction, such as the worker-queen conflict in the Hymenoptera (Trivers and Hare 1976) or conflict between nuclear and cytoplasmic genes (Werren and Beukeboom 1998). Overall, these theoretical studies have fueled many empirical studies, and together they have advanced our understanding of sex ratio or, more generally, of evolutionary patterns (Hardy 2002).

On the other hand, fewer studies have been done on mechanisms of sex-ratio variation. This is probably because of the belief that studying evolutionarily stable sex ratios does not require exact knowledge of the genetic background producing them. However, evolutionarily stable sex ratios are not independent of the underlying mechanisms although they are not fully constrained by the mechanism as evidenced by the presence of large sex-ratio variations under chromosomal sex determination (West and Sheldon 2002).

The sex-determining mechanism is one of the factors affecting sex ratios. However, other genetic or non-genetic factors such as sex-ratio genes, cytoplasmic sex factors, or environmental factors may also affect the sex ratio. On the other hand, the importance of sex determination lies not only in its relevance to sex ratios but also to the problem of sex itself. After all, what sex is and how sex is determined are two of the fundamental questions in biology. Recent studies have succeeded in identifying sex-determining genes (*Sry* in mammals, Sinclair *et al.* 1990; *DMY* in a fish, Matsuda *et al.* 2002). However, most information comes from a limited number of model organisms, and the wide variety of sex-determining mechanisms in many organisms have not been studied. One of the few exceptions is the insightful work on sex-determining mechanisms and its evolution by Bull (1983).

Experimental studies on sex ratio are generally easy to conduct. After all, to study the sex ratio of a population one has only to count the numbers of males and females at an appropriate stage of the life cycle. Considering this, it is surprising how few studies have been done to elucidate the genetic background that produces sex-ratio bias in organisms other than vertebrates and insects. The Mollusca—the most diverse animal taxon in terms of both the number of species and the modes of life, except for the Arthropoda—is no exception. To date, studies on the genetic mechanism

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producing various sex ratios in the Mollusca are limited to only a few groups, such as the oysters of the genus *Crassostrea* Sacco, 1897 (Haley 1977, Guo *et al.* 1998), the mussels of the genus *Mytilus* Linnaeus, 1758 (Saavedra *et al.* 1997, Kenchington *et al.* 2002), and the apple snail *Pomacea canaliculata* (Lamarck, 1822) (Yusa and Suzuki 2003, Yusa 2004b, 2006, 2007).

The purpose of this paper is to review studies on the mechanisms that produce sex ratios and the modes of sex determination in molluscs. I do not treat the adaptive significance of sex ratios in detail as there are many good papers such as Hamilton (1967), Charnov (1982), and Hardy (2002).

## DEFINITION OF SEX RATIO

I define the sex ratio as the proportion of males / (males + females). I consider the sex ratio in populations consisting mainly of male and female individuals. Thus, in this review I include sex-changing molluscs, as each individual is either male or female at a time. I do not consider simultaneous hermaphrodites, except for treating them as a factor biasing the sex ratio. Charnov (1982) provides the theoretical framework of sex allocation in simultaneous hermaphrodites. Studies on sexual issues in simultaneously hermaphroditic molluscs are reviewed in Leonard (1991), Baur (1998), and other contributions from this symposium.

Sex ratios can be measured at different stages of the life cycle of an organism. Sex ratio at fertilization or when sex is determined is called the primary sex ratio. Sex ratio at birth is called the secondary sex ratio. Sex ratios at later stages can also be considered, such as sex ratio at sexual maturity or that of individuals available for mating (operational sex ratio; Emlen and Oring 1977). In this review, I mainly treat the primary sex ratio. Other sex ratios are referred to as "apparent sex ratios."

Sex ratios can be considered at the population level (population sex ratio) or within each brood (brood or offspring sex ratio). These two are not always the same. For instance, in infinite populations, parents producing any brood sex ratios are equally adaptive although population sex ratio will become 0.5 by Fisherian selection (Williams 1979).

## MECHANISMS THAT MAY AFFECT APPARENT SEX RATIOS

Data on sex ratios are often taken from a field population as the proportion of males in adult individuals, without any information on sex differences in mortality, size, or other features. Thus, almost inevitably, many factors potentially affect the apparent sex ratio. To determine the primary sex ratio, well-controlled experiments, where confounding factors are eliminated, are ideal. However, it is often impossible to conduct such experiments, or the field data themselves may be the goal of a study (Takeuchi *et al.* 2007). Even so, many factors may potentially bias the apparent sex ratio (Table 1).

### Misidentification of sex

Many molluscs show sexual dimorphism in (i) the shape of the shell or the soft parts (some unionoids, Dillon 2000; the ampullariids *Marisa cornuarietis* [Linnaeus, 1758], Demian and Ibrahim 1972; and *Pomacea canaliculata*, Cazzaniga 1990); (ii) body color (the ampullariid *Marisa cornuarietis*, Demian and Ibrahim 1972); or (iii) body size (ampullariids and viviparids, Dillon 2000; assimineid snails *Assiminea japonica* Martens, 1877 and *Angustassiminea castanea* [Westerlund, 1883], Kurata and Kikuchi 2000). In such cases, the sex may be identified by external morphology without sacrificing the animals. However, the external morphology is often unreliable in identifying sex, and inspection of the gonads or other reproductive organs is preferable whenever possible. Even if gonads are examined, misidentification may occur, especially when only a small amount can be excised for inspection to keep the animals alive (Bauer 1987).

### Various sampling biases

Males and females may differ in habitat use, behavior (such as mobility and activity patterns), conspicuousness (in terms of color or brightness), or body size. For instance, in

**Table 1.** Mechanisms that may affect apparent and primary sex ratios.

1. Mechanisms for apparent sex ratios
    - Misidentification of sex
    - Sampling biases due to differential habitat use, behavior, etc.
    - Differential mortality (embryonic, juvenile, or later)
    - Differential age at maturity
2 Mechanisms for primay sex ratios
    - Sex-ratio genes and cytoplasmic factors
    - Sexual system
        - Parthenogenesis
        - Sex change
        - Simultaneous hermaphroditism
    - Mode of sex determination
        - Environment
        - Sex-determining genes
            - Heterogamety
            - Oligogenes
            - Polygenes

the viviparid *Viviparus ater* (Cristofori and Jan, 1832), females tend to hibernate earlier than males; thus, the apparent sex ratio of the population in autumn is male-biased (Keller and Ribi 1993). Different habitat use has been suggested as a cause of female-biased sex ratios in another viviparid, *Sinotaia quadrata historica* (Gould, 1859) (Hirai *et al.* 2004). In many snails females grow larger than males (*e.g.*, *Pomacea canaliculata*, Cazzaniga 1990; *Assiminea japonica*, Kurata and Kikuchi 2000) although in some species males are larger (*Angustassiminea castanea*, Kurata and Kikuchi 2000). Sexual dimorphism in size affects the apparent sex ratio if researchers sample larger individuals more often than smaller ones.

### Differential mortality

The sex ratio at birth may be different from the primary sex ratio if the hatching rate differs between the sexes. This effect is especially important when the hatching rate is low. However, the effect of differential hatching rate among egg masses can be assessed by studying the correlation between hatching rate and the secondary sex ratio. If there is no significant correlation, then the differential hatching rates are not responsible for the sex-ratio variation among egg masses (Yusa and Suzuki 2003).

Differential mortality in juvenile or adult stages may also skew the sex ratio at later stages (*e.g.*, *Sinotaia quadrata historica*, Hirai *et al.* 2004; *Busycon carica* [Gmelin, 1791], Avise *et al.* 2004). In addition, if age at maturity differs between the sexes, the sex that matures earlier will normally outnumber the sex that matures later due to the higher mortality of the latter before reaching sexual maturity.

## MECHANISMS RESPONSIBLE FOR PRIMARY SEX RATIOS

There are three general categories of mechanisms that may affect primary sex ratios (Table 1): sex-ratio genes and cytoplasmic factors, the sexual system (gonochoric, hermaphroditic, or parthenogenetic), and the mode of sex determination.

### Sex-ratio genes and cytoplasmic factors

Sex-ratio genes are nuclear genes that are expressed in the parents (the father, the mother, or both) and control the sex ratio of the offspring. For example, X-chromosome drive genes in *Drosophila* skew the proportion of X-carrying sperm during meiosis or fertilization (Hamilton 1967, Stouthamer *et al.* 2002).

Cytoplasmic sex factors or distorters are genetic elements present in the cytoplasm, such as the bacterial genus *Wolbachia* (Stouthamer *et al.* 2002). They often distort the host's sex ratio towards female, because the parasite is usually inherited only through the female lineage and hence female-biased sex ratios are advantageous to them. Cytoplasmic sex factors are not known in Mollusca, and a preliminary trial to detect them was unsuccessful in *Pomacea canaliculata* (Yusa 2006). This does not necessarily mean, however, that all molluscs are free from these factors. A possible candidate, for example, is the paternally inherited (M) mitochondria of *Mytilus* spp. (Saavedra *et al.* 1997, Sutherland *et al.* 1998, Zouros 2000, Kenchington *et al.* 2002, Cao *et al.* 2004) and unionoid bivalves (Dillon 2000).

### Sexual system

#### *Parthenogenesis*

Molluscs with highly female-biased sex ratios often turn out to reproduce parthenogenetically. For instance, in the freshwater snail *Potamopyrgus antipodarum* (Gray, 1843), the sex ratio varies from 0-50% among populations (Wallace 1992). In most populations the sex ratio is female-biased (Lively 1992). The populations with extremely low sex ratios consist of triploid females that reproduce parthenogenetically, and populations with ratios that are approximately 0.5 consist of diploid sexuals (Lively 1992, Wallace 1992). In some populations, parthenogenetic and sexual individuals coexist; these populations have intermediate sex ratios (Lively 1992, Jokela *et al.* 1997).

Freshwater clams of the genus *Corbicula* Megerle von Mühlfeld, 1811 are predominantly hermaphrodites, reproducing by self-fertilization. However, maternal genes are extruded from the oocyte during the first meiotic division, so that fertilized eggs have only the paternal nuclear genome (androgenesis; Komaru *et al.* 1997, Ishibashi *et al.* 2003). Because the offspring have the same genome as the parent through non-reductional sperm, this represents a special case of parthenogenesis.

#### *Sex change*

Sex change is either protandrous (first mature as male and then change sex to female) or protogynous (female to male). The occurrence of sex change has two major effects on sex ratio. First, the brood sex ratio, when followed as a time series, is biased towards the first-maturing sex when they are young, and then skews towards the later-maturing sex as a direct consequence of sex change, as shown in *Crassostrea gigas* (Thunberg, 1793) (Guo *et al.* 1998). Secondly, sex change affects the sex ratio of the population as well. The sex ratio should be distorted towards the first developing sex in sex changers (Charnov and Bull 1989). In fact, several studies have reported male-biased sex ratios in protandrous molluscs (the oysters *Crassostrea* spp., Haley 1977, Guo *et al.* 1998; the pearl oyster *Pinctada mazatlanica* Jameson, 1901, Arnaud-Haond *et al.* 2003; the slipper shell of the genus

*Crepidula* Lamarck, 1799, Hoagland 1978, Collin 1995, Richard *et al.* 2006).

Sex ratio may vary seasonally or spatially in protandrous molluscs. For instance, in *Crepidula convexa* Say, 1822, the sex ratio is male-biased from fall to spring, when new recruits become sexually mature as males, then female-biased in summer when many of them change sex to female (Hoagland 1978). Hoagland also reported positive correlations between adult density and sex ratio in many local populations of *Crepidula fornicata* (Linnaeus, 1758) and *C. convexa*. This is not due to adaptive sex-ratio adjustment in response to variable density, but rather to variation in the number of recruits among sites: sites with many recruits have higher densities and, after the recruits mature as males, have higher proportions of males.

### *Simultaneous hermaphroditism*

In some organisms, simultaneous hermaphrodites coexist with males (androdioecy), females (gynodioecy), or both (trioecy). In the Mollusca, coexistence of gonochoric individuals and simultaneous hermaphrodites has been reported (unionoid bivalves, Dillon 2000; the freshwater pearl mussel *Margaritifera margaritifera* [Linnaeus, 1758], Bauer 1987; *Mytilus* spp., Saavedra *et al.* 1997). When the proportion of hermaphrodites is small and the population consists mainly of gonochoric individuals, the sex ratios are nearly 0.5 in most species studied so far (for unionoid bivalves, see table 2.2 in Dillon 2000; in *Mytilus*, Saavedra *et al.* 1997). In the freshwater mussel, *Margaritifera margaritifera*, the proportion of males in the population consisting of males, females, and hermaphrodites is nearly 0.5 (Bauer 1987). Because females can change sex to hermaphrodites and *vice versa*, the equal proportion of males and non-males suggests that a simple genetic mechanism such as heterogamety is involved (Bauer 1987).

## MODE OF SEX DETERMINATION

### Environmental sex determination

In the case of gonochoric organisms, an individual's sex is determined environmentally, genetically, or both. Environmental sex determination can result from factors such as temperature, food availability, daylength, or the presence of adult individuals (Bull 1983). Well-known molluscan examples are slipper shells of the genus *Crepidula*, in which small individuals that settle on larger individuals mature as males, whereas solitary ones mature as females (Hoagland 1978). These small males may change sex, but there are also true males which do not have the ability to change sex (Coe 1936; although Hoagland raises some doubt about this). As an adaptive response, sex ratios in organisms with environmental sex determination are expected to be biased towards the sex developing in the worse conditions (*e.g.*, poor nutrients; Charnov and Bull 1989).

### Genetic sex determination

Sex-determining genes can be distinguished by the number of genes into two-factor, oligogenic and polygenic systems. Sex determination by heterogamety, such as XY (male heterogamety) or ZW (female heterogamety), is a well-known system involving two genetic factors. The majority of gonochoric molluscs with known sex-determining systems are XY (gastropods, Nakamura 1986; some bivalves, Allen *et al.* 1986, Guo and Allen 1994). Examples of XO sex determination, with XX females and XO males, are widespread in the Neritidae (Nakamura 1986).

In species of the freshwater snails *Viviparus* Montfort, 1819, both XY and ZW sex determinations are known to occur, although ZW is the majority (Barsiene *et al.* 2000). This has an important implication that one sex-determining system (*e.g.*, XY) can evolve from another (ZW) relatively easily.

Under heterogamety, the expected brood and population sex ratios are both 0.5, with little variation expected under the binomial distribution unless other mechanisms affect the sex ratios. In accordance with this, the sex ratio is 0.5 in most molluscan populations where data are available (*e.g.*, in the unionoid bivalves and in freshwater "prosobranchs", Dillon 2000), although female-biased sex ratios are reported in some "prosobranchs" such as *Pomatiopsis cincinnatiensis* (Lea, 1840) and *Goniobasis* (also referred to as *Elimia* H. & A. Adams, 1854) *semicarinata* (Say, 1829) (Dillon 2000). Unfortunately, due to the lack of sufficient information, the reason for the female-bias is unknown.

Oligogenic sex determination depends upon a small number of genes. For instance, in the platyfish and wood lemming, sex is determined by three genetic factors, X, Y, and W (Bull 1983). Under oligogenic sex determination, offspring sex ratios vary among different pairs of parents. For instance, in the platyfish, mating between an XX female and a YY male produces all sons (XY males), whereas mating between a WX female and an XY male produces only one-quarter sons (Bull 1983).

Polygenic sex determination is a system in which many genes are involved in determining an individual's sex. Each gene has only a minor effect on sex determination, and hence, sex-ratio variation. Models of polygenic sex determination are proposed by Bulmer and Bull (1982) and Bull (1983). There are no molluscan examples of polygenic sex determination.

## SEX-RATIO VARIATION AND SEX DETERMINATION IN *POMACEA CANALICULATA*

The apple snail *Pomacea canaliculata* (Ampullariidae) is a South American freshwater snail introduced into many Asian countries, including Taiwan, the Philippines, Thailand, Vietnam, Japan, and China (Naylor 1996, Yusa and Wada 1999). It is a serious rice pest (Naylor 1996) as well as a keystone species controlling the function of wetland ecosystems (Carlsson *et al.* 2004). Because no effective control methods have been developed, sex-ratio variation in *P. canaliculata* has been studied to explore novel genetic control methods (Yusa 2004a, 2004b).

*Pomacea canaliculata* is gonochoric. Although there is a report that *P. canaliculata* can change sex (Keawjam and Upatham 1990), later authors have failed to confirm this. Sex ratios in populations in rice fields are often female-biased (Banpavichit *et al.* 1994, Tanaka *et al.* 1999), but these may be due to differential survival or growth rates between the sexes.

To study the sex ratio at birth, we removed up to 80 hatchlings from each egg mass, and reared them for 50 days or more, until individuals began developing their reproductive organs (Yusa and Suzuki 2003). We dissected all snails to identify the sexes based on the presence or absence of the testis (for males) or the albumen gland (for females). We used egg masses collected for three months (July, August, and September) in organic rice fields where no chemicals had been sprayed, and those laid by laboratory-reared pairs.

Sex ratios varied greatly among egg masses from nearly all males to nearly all females (Fig. 1). In many egg masses, the sex ratio was significantly different from 0.5. For instance, in July the sex ratio was significantly different ($P < 0.05$ by binomial test) from 0.5 in 15 out of 27 egg masses. This proportion is much higher than expected by chance (only 1 or 2 out of 27 egg masses are expected to be statistically significant at $P = 0.05$). Likewise, the sex ratio was significantly biased in 13 of 25 egg masses in August, and in 7 of 27 in September.

Irrespective of the large variation among egg masses, the average sex ratio was close to 0.5 in all three months (mean ± SD = 0.47 ± 0.20 in July; 0.53 ± 0.25 in August; and 0.47 ± 0.18 in September). Thus, brood sex ratios of *Pomacea canaliculata* were highly variable, yet population sex ratios were unbiased. We repeated similar experiments using egg masses of laboratory-reared mating pairs originating from two different populations. The results were nearly identical: large variations in brood sex ratios and unbiased population sex ratios were detected in all eight experiments conducted (Yusa and Suzuki 2003).

Survival was high (94-98%) during the rearing period and did not explain the variation in brood sex ratio. Hatching rate was low, but there was no correlation between hatching rate and sex ratio in any experimental series. A possible factor responsible for variation in sex ratio was egg weight. The relationship between the average weight of an egg and the sex ratio was negative in all eight experimental series, and the relationship was significant in three experimental series. Heavier eggs tended to produce female snails whereas lighter ones produced males. Although the meaning of this relationship is unknown, the effect was too small to explain all of the variation.

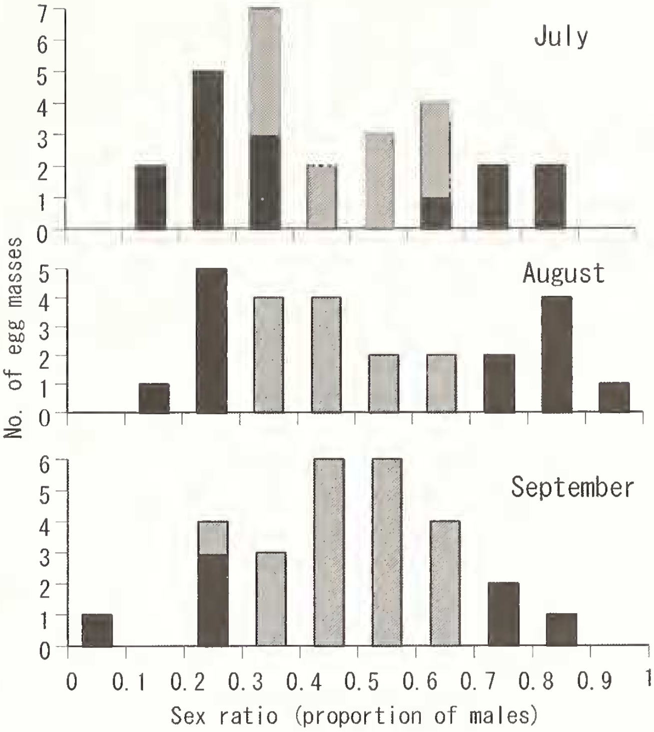

**Figure 1.** Brood sex ratio (proportion of males) of field-collected egg masses in the apple snail *Pomacea canaliculata* (after Yusa and Suzuki 2003). Black areas indicate egg masses whose sex ratios are significantly different from 0.5 ($P < 0.05$ by binomial test); gray areas indicate those that are not significant.

### Environmental effects on sex ratio in *Pomacea canaliculata*

To study the effects of environment on brood sex ratio, I used a split-brood design, where each brood was split into two (or sometimes three) groups of nearly equal numbers of hatchlings, and each group was reared under two different conditions for each environmental factor (Yusa 2004b). I tested factors such as the presence of adult males or females, food availability, temperature, age of the parents, size of the aquarium (as an index of crowding), and indoor or outdoor conditions (with different daylengths).

Among the environmental factors studied, none had a significant effect on brood sex ratio. On the other hand, brood sex ratios were significantly different among different egg masses or parents in all experiments. These results suggest that environmental sex determination does not occur and that the variation in sex ratio is under genetic influence in this species.

### Genetics of sex-ratio variation in *Pomacea canaliculata*

To investigate the genetics of sex-ratio variation, I studied parent-offspring regressions and sib correlations of sex ratio (Yusa 2006). For parent-offspring regressions, the sex ratio of each brood was regressed to the sex ratio of its father's siblings or that of its mother's siblings. Correlations in sex ratios between sisters and between brothers were also investigated.

There were significant positive relationships in brood sex ratio in the female lineage: the regression between offspring sex ratio and the sex ratio of the mother's siblings (slope = 0.28) and the correlation between the offspring sex ratios of two sisters ($r = 0.41$) were both significant ($P < 0.05$). On the other hand, father-offspring regression (slope = 0.10) and the correlation between two brothers ($r = -0.13$) were not significant (Yusa 2006).

Thus, the results suggest inheritance of sex-ratio variation through the female lineage. However, the regression or correlation coefficients were too low to postulate cytoplasmic sex factors or maternal genes, which may show coefficients of nearly 1.0 for mother-offspring regression and correlation between sisters (Table 2; Yusa 2006). The results were not congruent with typical sex-ratio genes or sex-determining polygenes with additive effects, which will show coefficients of nearly 0.5 for both male and female lineages (Table 2). A further study suggests that the variation in sex ratio is due to sex determination by a small number of genes (oligogenic sex determination; Yusa, 2007).

Oligogenic sex determination means the coexistence of multiple sex-determining genes in a population. It may occur during the evolutionary transition from one sex-determining system to another, or it may result from a mixture of two or more populations with different sex-determining systems. In *Pomacea canaliculata*, XY sex determination is reported in some Japanese populations (von Brand *et al.* 1990) but not in Brazil (Mercado Laczkó and Lopretto 1998). This discrepancy suggests that different sex-determining systems may coexist in *P. canaliculata*, as they do in the frog *Rana rugosa* Schlegel (Nishioka *et al.* 1994).

## GENETICS OF SEX DETERMINATION IN OTHER MOLLUSCS

### Sex determination in *Crassostrea*

Oysters of the genus *Crassostrea* change sex. In general, they are protandrous hermaphrodites, but the details of the sexual system differ among species and experimental conditions.

In *Crassostrea virginica* (Gmelin, 1791), the majority of individuals change sex from male to female, but a small proportion of individuals may change from female to male (Haley 1977). Pure males and pure females also exist. Haley proposed an additive 3-loci model to explain the difference in brood sex ratio and patterns of sex between families, with *m* being the allele for maleness and *f* for femaleness. Under this hypothesis the sex of an individual is determined by ratio of *m:f* alleles, such that those with 3-6 *m*'s are true males, those with 2 *m*'s are protandrous hermaphrodites, 1 *m* are females (possibly protogynous hermaphrodites), and no *m* are true females. To my knowledge, this is the only study suggesting oligogenic sex determination in the Mollusca. However, both the experiments and the hypothesis have been criticized by later authors (Guo *et al.* 1998; Yusa and Suzuki 2003).

In *Crassostrea gigas*, individuals are either protandrous hermaphrodites or true males. In a study of parental effects on the sex ratio, Guo *et al.* (1998) found that the sex ratio of

**Table 2.** Expected regression or correlation coefficients of brood sex ratios under various genetic systems and those actually obtained in *Pomacea canaliculata* (after Yusa 2006). The genes are supposed to have additive effects.

| Genetic system | Mother-offspring regression | Father-offspring regression | Correlation between sisters | Correlation between brothers |
|---|---|---|---|---|
| Cytoplasmic sex factors | 1 | 0 | 1 | 0 |
| Sex-ratio genes (biparental) | 0.5 | 0.5 | 0.25 | 0.25 |
| Polygenic sex determination | 0.5 | 0.5 | 0.25 | 0.25 |
| Data obtained in *P. canaliculata* (mean ± SE) | 0.28 ± 0.13 | 0.10 ± 0.16 | 0.41 ± 0.18 | −0.13 ± 0.19 |

individuals at one year of age was dependent on the father and independent of the mother. They proposed that the sex is determined by a single locus, with *M* and *F* genes, and individuals of *MF* genotype become males and those with *FF* genotype are protandrous hermaphrodites.

### Sex-ratio variation and doubly uniparental inheritance of mitochondria in *Mytilus* spp.

In the mussels *Mytilus edulis* Linnaeus, 1758, *Mytilus trossulus* (Gould, 1850), and *Mytilus galloprovincialis* Lamarck, 1819, brood sex ratios vary from all males to all females (Saavedra *et al.* 1997, Kenchington *et al.* 2002). Irrespective of this variation, the average of brood sex ratios (population sex ratio) is nearly 0.5 (Saavedra *et al.* 1997, Zouros 2000, Kenchington *et al.* 2002). Such highly variable brood sex ratios under equal population sex ratios are similar to the sex-ratio patterns in *Pomacea canaliculata*. The major difference between the mussels and the apple snail is that in the mussels sex ratio appears to be dependent only on the mother and independent of the father (Saavedra *et al.* 1997, Zouros 2000, Kenchington *et al.* 2002; but see below), whereas in the apple snail both parents contribute equally to the sex-ratio variation (Yusa 2007).

In mussels, sex-ratio variation involves a phenomenon called "doubly uniparental inheritance" (DUI) of mitochondria (Saavedra *et al.* 1997, Zouros 2000, Kenchington *et al.* 2002). There are two types of mitochondria, M and F. The M types are transmitted only from the father to the sons through sperm, and the F types from the mother to both sons and daughters through eggs. DUI has been demonstrated in five families of bivalves: Mytilidae, Unionidae, Hyriidae, Margaritiferidae, and Veneridae (Kenchington *et al.* 2002, Cogswell *et al.* 2006, Walker *et al.* 2006).

Saavedra *et al.* (1997) postulate a masculizing factor, W, in M-type mitochondria. This is supported by the observations that (i) some fathers who fail to pass the M genome to their offspring have few sons and many daughters (Saavedra *et al.* 1997), and that (ii) M mitochondria are passed into female embryos as well as male embryos. However, in the female embryos they appear to be outnumbered by F mitochondria whereas in the male embryos M mitochondria preferentially enter into the supposed germ line (Sutherland *et al.* 1998, Cao *et al.* 2004, Cogswell *et al.* 2006). These observations suggest that embryos with many M types in the germ line will develop the testis and become males. Because some fathers fail to pass the M genome to their offspring, at least some part of genetic sex-ratio variation depends on the father. However, this effect is small and the major source of sex-ratio variation depends on the mother (Saavedra *et al.* 1997, Kenchington *et al.* 2002).

The fact that most genetic variation in the sex ratio depends on the mother suggests that some factor from the mother controls the behavior of M mitochondria. Saavedra *et al.* (1997) postulate that there is a feminizing factor Z, and the amount of the factor in the eggs depends on the mother. Kenchington *et al.* (2002) further postulate that the factor is controlled by a pair of alleles (*Z* and *z*) at a single locus. This allows only three genotypes of females: *ZZ*, *Zz*, and *zz*. The *ZZ* mothers produce almost all sons, *Zz* mothers produce both sons and daughters at a fixed ratio, and *zz* mothers produce all daughters (Kenchington *et al.* 2002). However, the simple 2-factor model of a feminizing factor Z does not fully explain the variable brood sex ratios observed in *Mytilus*. Continuous but highly variable sex ratios probably require more factors, at least some of which may be genetic, as suggested by the pedigree experiments (Kenchington *et al.* 2002).

In summary, there appears to be a masculizing factor W in M mitochondria in *Mytilus* spp. Some genetic variation exists in the fathers' ability to pass the M genome to the offspring. Also there may be another sex-determining factor Z in the eggs, which may control the action of W. The genetics of Z factor is unknown, but probably more than two genes or alleles are necessary to explain the large variation in the sex ratio. The genetics of sex-ratio variation in *Mytilus* therefore seems to be oligogenic.

## CONCLUSION AND FUTURE DIRECTION

Sex ratio is a basic property of a population and hence has direct relevance to the fitness of the individual controlling the sex ratio of the brood. The sex-determining system may not be fixed, but rather may be fairly labile and subject to evolutionary changes. For example, XY and ZW sex determination coexist in *Viviparus*; there appears to be oligogenic sex determination in *Pomacea canaliculata*, and possibly in *Mytilus* spp.

Studies on variation in sex ratio, such as the ones on *Pomacea canaliculata*, are very easy to conduct—just rear the hatchlings and determine their sexes. Also, studies on sex chromosomes are not difficult, yet very few studies have been done in molluscs, especially outside gastropods. Considering the wide variety of molluscan taxa and their life histories, I suspect that studies may lead to unanticipated findings.

At the same time, novel techniques are useful. For instance, Avise *et al.* (2004) developed molecular sex identification techniques using microsatellite loci in the whelk *Busycon carica*. Also, artificial formation of triploid individuals by cytochalasin B treatment was successful in elucidating detailed mechanisms of XY sex determination, such as Y being dominant to X in the dwarf surfclam *Mulinia lateralis* (Say, 1822) (Guo and Allen 1994), or sex determination by X:autosome ratio in soft-shelled clam *Mya arenaria* (Allen *et al.* 1986). Such techniques will be powerful tools to study the genetics of sex determination and sex-ratio variation in molluscs.

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